

## Chapter 2

# Fifty Years of Progress in Drug Metabolism and Toxicology: What Do We Still Need to Know About Cytochrome P450 Enzymes?

F. Peter Guengerich

**Abstract** The 50 years following the discovery of the cytochrome P450 system have been years of remarkable progress in the basic science and in its application to important problems, particularly in medicine. This chapter reviews what has been done, at both basic and applied levels. My own views on the still unresolved basic issues are presented, along with some thoughts about opportunities for future development in practical applications.

**Keywords** Agricultural applications • Basis of pharmacokinetics • Catalytic mechanisms • Clinical applications • Cytochrome P450 • Drug metabolism • Endocrinology • Metabolic diseases • Metabolism of carcinogens • Processivity of P450 reactions • Toxicology

## 2.1 Introduction

I wish to begin this chapter with two personal notes, in celebrating the 50th anniversary of the first real report of cytochrome P450 (P450). One is a round of thanks to and acknowledgment of Professor Tsuneo Omura, who co-authored the original paper in *The Journal of Biological Chemistry* (Omura and Sato 1962) 50 years ago as a graduate student with his mentor Professor Ryo Sato. He is still a brilliant but humble man who follows the field and attends the meetings. The other note is that I entered the P450 field as a postdoctoral fellow with

---

F.P. Guengerich (✉)

Department of Biochemistry and Center in Molecular Toxicology, Vanderbilt University  
School of Medicine, 638 Robinson Research Building, 2200 Pierce Avenue, Nashville,  
TN 37232-0146, USA  
e-mail: [f.guengerich@vanderbilt.edu](mailto:f.guengerich@vanderbilt.edu)

Professor Minor J. Coon in 1973, four decades ago at the time I finished this article. I had no idea that I would continue to work on this same enzyme system for 40 years. I work on other enzyme and nucleic acid systems too, but there have just been too many interesting things to do with P450 and I do not think I can ever stop.

The field of P450 research began, in part, with individuals who were interested in a liver protein with unusual spectral properties (Garfinkel 1958; Klingenberg 1958; Omura and Sato 1962, 1964a, b). However, what has driven research in the P450 field has been its relevance to a number of areas. Reactions that we now know are catalyzed by P450s were already described in chemical carcinogenesis (Mueller and Miller 1948, 1953), drug metabolism (Gillette et al. 1957), and steroid metabolism (Ryan 1958) when the pigment with the unusual spectrum was identified. Key studies along the way were the carbon monoxide inhibition studies establishing the newly found P450 as the terminal oxidase in microsomal electron transport (Estabrook et al. 1963), several lines of investigation suggesting the existence of multiple P450s (Alvares et al. 1967; Hildebrandt et al. 1968; Sladek and Mannering 1969), and the separation and reconstitution of the components of the microsomal P450 system (Lu and Coon 1968) (see also the chapter by Professor Tsuneo Omura in this monograph). Another notable contribution was the work of Professor Irwin Gunsalus and his associates (Katagiri et al. 1968) with the bacterial model P450<sub>cam</sub> (CYP101A1), which provided interesting biophysical insights into the structures (Poulos et al. 1985) and catalytic mechanisms (Tyson et al. 1972) for both bacterial and mammalian P450s (Mueller et al. 1995; McLean et al. 2005).

The field of P450 research is still fueled by its relevance in the fields of metabolism in chemical carcinogenesis, drug metabolism, and endocrinology. However, there are also many practical applications in medicine (Nebert and Russell 2002), nutrition (Plum and DeLuca 2010), agriculture (Mizutani and Sato 2011), and biotechnology, including the use of P450s as designed catalysts (Guengerich 2002; Coelho et al. 2013). The number of academic research papers on P450 continue to increase each year, and more than 33,000 papers related to P450 research have been published (<http://webtools.mf.uni-lj.si/public/medsum.html>) with about 2,000 per year still being added.

## 2.2 What We Know About P450

The following list is not intended to be comprehensive but is a summary of what I consider the most important. Even with these there are some missing pieces of information.

First of all, the number of P450 genes is now known in many organisms, including humans (57) (Table 2.1). Defining these numbers was not trivial, and the final answers really came with the completion of genomic sequences. It is of note that some microorganisms have a fairly high number of P450s (e.g., 32 in *Streptomyces avermitelus*) and plants have hundreds.

**Table 2.1** Classification of human P450s based on major substrate class

Sterols	Xenobiotics	Fatty acids	Eicosanoids	Vitamins	Unknown
1B1 <sup>a</sup>	1A1 <sup>a</sup>	2J2	4F2	2R1 <sup>a</sup>	2A7
7A1 <sup>a</sup>	1A2 <sup>a</sup>	4A11	4F3	24A1 <sup>a</sup>	2S1
7B1	2A6 <sup>a</sup>	4B1	4F8	26A1	2U1
8B1	2A13 <sup>a</sup>	4F12	5A1	26B1	2W1
11A1 <sup>a</sup>	2B6 <sup>a</sup>		8A1 <sup>a</sup>	26C1	3A43
11B1	2C8 <sup>a</sup>			27B1	4A22
11B2	2C9 <sup>a</sup>				4F11
17A1 <sup>a</sup>	2C18				4F22
19A1 <sup>a</sup>	2C19 <sup>a</sup>				4V2
21A2 <sup>b</sup>	2D6 <sup>a</sup>				4X1
27A1	2E1 <sup>a</sup>				4Z1
39A1	2F1				20A1
46A1 <sup>a</sup>	3A4 <sup>a</sup>				27C1
51A1 <sup>a</sup>	3A5				
	3A7				

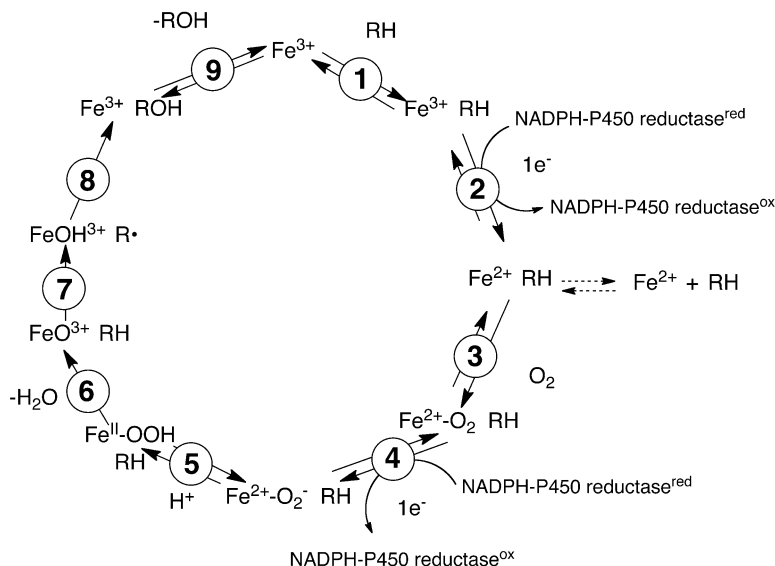
<sup>a</sup>X-ray crystal structure(s) reported (for human enzymes)<sup>b</sup>Bovine X-ray crystal structure reported (Zhao et al. 2012)

Source: Guengerich (2005)

Another success, which may not be appreciated today, is definition of the number of different proteins involved in P450 function. Bacteria have a myriad of different redox systems (Guengerich and Munro 2013). Mammalian microsomal P450s all accept electrons from NADPH-P450 reductase (POR), with cytochrome *b*<sub>5</sub> being involved in some but not all P450 reactions. Mammalian mitochondrial P450s use an electron-transfer system related to some bacteria, involving the iron–sulfur protein adrenodoxin and the flavoprotein NADPH-adrenodoxin reductase. Although the literature contains early (Nelson et al. 1973) and more recent (Hughes et al. 2007) reports of other proteins being involved, the current literature has not confirmed the roles of any of these.

### 2.2.1 Electron Transfer

The source of reducing equivalent for P450s, with only a few exceptions (Guengerich and Munro 2013), is NAD(P)H. Pyridine nucleotides are two-electron donors and, with one exception (Shiro et al. 1995), do not reduce P450, a one-electron acceptor. Flavoproteins are biological transformers and can be involved in both one- and two-electron transfers. With microsomal P450s, electrons come from POR, a two-flavin protein, in a system where electrons flow from NADPH to FAD to FMN then to P450s. In some cases cytochrome *b*<sub>5</sub> donates the “second” electron (to the FeO<sub>2</sub><sup>2+</sup> complex). For the seven mitochondrial P450s [11A1, 11B1, 11B2, 24A1, 27A1, 27B1, 27C1 (note: 27C1 is tentative)], electrons flow from NADPH to the flavoprotein NADPH-adrenodoxin reductase to adrenodoxin to the P450.



**Fig. 2.1** General catalytic cycle for P450 reactions (Guengerich 2001)

Rates of electron transfer to many of the human P450s have been measured (Guengerich and Johnson 1997). Some are dependent upon the binding of a substrate to the P450, but this is not always the case (Guengerich and Johnson 1997). However, the concentration of total P450 is about 20 fold higher than POR in the liver (Estabrook et al. 1971). Reduction rates are usually biphasic in microsomes, with the P450 in closest proximity being reduced first (Peterson et al. 1976).

Cytochrome  $b_5$  can provide the “first” electron (to ferric P450), but the rate is slow, probably because of the unfavorable redox potential (West et al. 1974; Yamazaki et al. 1996). Such a functional system can apparently occur *in vivo* as well (Henderson et al. 2013). Measurement of the rate of cytochrome  $b_5$  electron transfer to the P450  $\text{FeO}_2^{2+}$  complex is technically difficult and has only been done in a few settings (Yun et al. 2005; Zhang et al. 2007).

### 2.2.2 Basic Catalytic Mechanism

The activation of oxygen to a reactive form is complex and involves unstable high-valent iron intermediates that have been difficult to study (Fig. 2.1). The subject has been discussed at length elsewhere (Ortiz de Montellano and De Voss 2002; Ortiz de Montellano and De Voss 2005).

The key species is the perferryl oxygen complex,  $\text{FeO}^{3+}$ . This complex is formed in the indicated pathway (Fig. 2.1) and then abstracts a hydrogen atom to leave a carbon-centered radical. “Rebound” of oxygen from the resulting  $\text{FeOH}^{3+}$  species

forms an alcohol (or an equivalent product). One variation on this theme involves the abstraction of a non-bonded electron from a low redox potential substrate (e.g., nitrogen), followed by possible rearrangements and then an oxygen rebound (Guengerich 2001; Ortiz de Montellano and De Voss 2005).

The only other viable oxidant at present is the  $\text{FeO}_2^-$  species, a precursor of  $\text{FeO}^{3+}$ . This nucleophilic species can explain some P450 reactions with aldehydes (Akhtar et al. 1982). The possibility exists that even some of those reactions might also have at least a partial contribution from a  $\text{FeO}^{3+}$ -based mechanism (Hackett et al. 2005).

Using these basic mechanisms, it has been possible to rationalize almost all the reported P450 reactions, with the inclusion of rearrangements of enzyme intermediates or reaction products (Guengerich 2001; Ortiz de Montellano and De Voss 2005; Isin and Guengerich 2007; Guengerich and Munro 2013; Guengerich and Isin 2014).

### 2.2.3 Multiple Rate-Limiting Steps

In early P450 research, there was a quest to find the “rate-limiting step” in the P450 reaction cycle (Diehl et al. 1970; Gigon et al. 1969). The subject has been addressed many times, utilizing pre-steady-state kinetics and kinetic isotope effect studies. There is evidence that steps **2**, **4**, **7**, and **9** in Fig. 2.1 can all contribute to rate determination (Guengerich 2013).

Of course, some of the reactions in Fig. 2.1 are difficult to measure (e.g., **3**, **5**, **6**, **8**), and rates are not known but are assumed to be fast. Two other points can be made. One is that the overall rate of a P450 reaction can be a function of the frequency of uncoupling (i.e., diversion of intermediate species to reduction of  $\text{O}_2$  to  $\text{O}_2^-$ ,  $\text{H}_2\text{O}_2$ , and  $\text{H}_2\text{O}$ ) (Gorsky et al. 1984). The other point is that even substrate binding (step **1** in Fig. 2.1) can be a complex, multi-step pathway moving the substrate from the periphery of the P450 to the active site (Isin and Guengerich 2006; Sevrioukova and Poulos 2012).

### 2.2.4 Regulation of Expression

In retrospect, early work in this area was extremely difficult because of the lack of appropriate technology and reagents. Today we recognize that most of the regulation of the P450 genes is at the transcriptional level. The general model follows that developed for steroid nuclear receptors: a cytosolic receptor binds a ligand, heterodimerizes with a partner protein, and moves to the nucleus. The loaded heterodimer binds to a specific (“consensus”) site in the 5′-regulatory sequence (“enhancer”) and alters the gene/chromatic structure to open the promoter region for RNA polymerase to copy the P450 gene faster. Indeed, several of the major receptors involved in P450 gene regulation were in the steroid nuclear receptor “orphan” group (e.g., PXR, CAR, PPAR $\alpha$ ). The well-known AhR/ARNT pathway for induction by polycyclic aromatic hydrocarbons, etc. follows a similar model (with unrelated proteins) (Williams et al. 2005).

Some regulation is more complex. The CAR pathway involves kinases and intersects with the epidermal growth factor receptor-signaling pathway (Mutoh et al. 2013). There are elements of posttranscriptional regulation in some systems, and microRNA regulation has been implicated in the regulation of P450s (Gomez and Ingleman-Sundberg 2009). Rodents show considerable gender-linked P450 regulation, which is the result of steroid, growth hormone, and STAT pathway regulation (Waxman and Holloway 2009) (this is not seen, at least at this level, in humans; Yang et al. 2010).

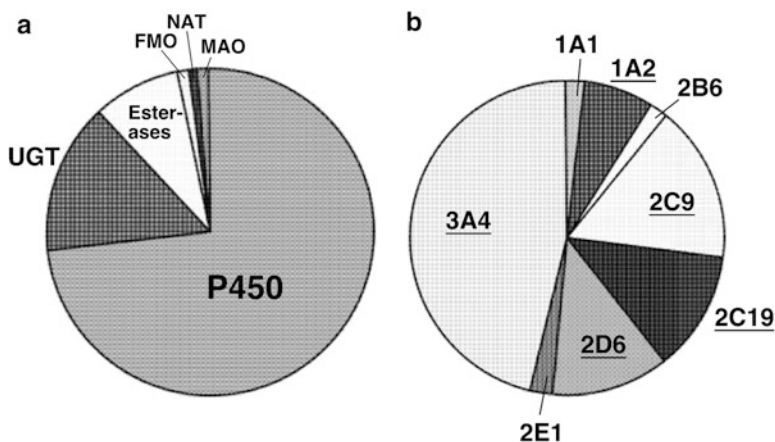
### 2.2.5 *Polymorphisms*

P450 differences among individuals have been suspected since early discoveries of individuals with inherited errors of metabolism in endocrine issues. These differences began to be characterized with the availability of recombinant DNA technology (White et al. 1984). The incidence of these variations is relatively low.

Polymorphisms are generally defined as variations in the population at a level greater than 1 % (Kalow 1962). One impetus for work in this area was the stratification of smokers into three groups based on the inducibility of aryl hydrocarbon hydroxylase (now associated with P450s 1A1 and 1B1) (Kellerman et al. 1973a, b). This area was driven by work by Professor Robert Smith and others, who found people (including Professor Smith himself) who could not effectively oxidize certain drugs (i.e., debrisoquine, sparteine, metoprolol) (Mahgoub et al. 1977). This “poor metabolizer” phenotype was consistent within individuals and showed Mendelian inheritance. Ultimately this phenomenon was understood in the context of P450 2D6 (Distlerath et al. 1985; Gut et al. 1986; Gonzalez et al. 1988). Today we know that there are not only two genotypes of P450 2D6 but more than 100. This situation is not atypical for the P450s, and today the collected genotypes are collected and available online ([www.cypalleles.ke.se](http://www.cypalleles.ke.se)). Today this variability of P450s and its effects on drug metabolism are a major component of “personalized medicine” (Evans and McLeod 2003).

### 2.2.6 *Cellular Localization*

Classically, mammalian P450s have been considered to be either microsomal (i.e., in the endoplasmic reticulum) or mitochondrial. Human P450s 11A1, 11B1, 11B2, 24A1, 27A1, 27B1, and (probably) 27C1 are considered to be mitochondrial. However, work by Prof. Narayan Avadhani has shown that fractions of some of the microsomal P450s can be localized in the mitochondria (Niranjan and Avadhani 1980). In at least some cases this localization is the result of cryptic import signals, which can be manifested by proteolytic cleavage (Addya et al. 1997). Some are sensitive to phosphorylation (e.g., P450 2E1) (Bansal et al. 2010), and polymorphisms in human P450s can determine the



**Fig. 2.2** Drug metabolism reactions. (a) Contributions of different enzymes. *UGT* UDP glucuronosyl transferase, *FMO* flavin-containing monooxygenase, *NAT* *N*-acetyltransferase, *MAO* monoamine oxidase. (b) Contributions of individual human P450 enzymes to (P450) drug metabolism (Williams et al. 2004)

partitioning between the endoplasmic reticulum and mitochondria, for example, P450 2D6 (Bajpai et al. 2013). Interestingly, the P450s that locate in the mitochondria can efficiently utilize the adrenodoxin electron-transport pathway in their function.

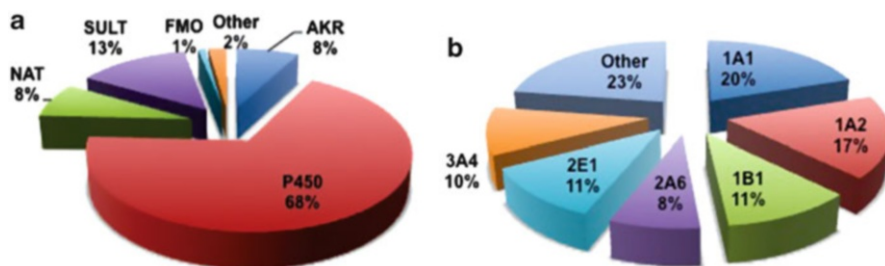
### 2.2.7 Roles of P450s in Individual Reactions

Determination of which P450s are involved in reactions is now a relatively straightforward *in vitro* process. Selective inhibitors, antibodies, comparisons with established markers, and purified P450s render the procedures very direct, given appropriate consideration of levels of expression (Beaune et al. 1986; Guengerich and Shimada 1991). Accordingly, there is extensive information about the human P450 regarding drug substrates, inhibitors, and inducers (<http://medicine.iupui.edu/clinpharm/ddis/main-table/>) (Fig. 2.2) and also carcinogen substrates (Rendic and Guengerich 2012) (Fig. 2.3).

### 2.2.8 Importance of P450s in Medical Practice

The significance of P450 research in medicine is considerable, as seen in several areas.

**Endocrinology** Steroid metabolism is complex, and many inborn errors of metabolism can now be explained in terms of deficiencies of individual P450s or NADPH-P450 reductase (Miller and Auchus 2011). Included among these are the



**Fig. 2.3** Carcinogen activation by human enzymes. (a) Contributions of different (human) enzyme systems. *FMO* flavin-containing monooxygenase, *NAT* *N*-acetyltransferase, *SULT* sulfotransferase, *AKR* aldo-keto reductase, *COX* cyclooxygenase/prostaglandin synthase. (b) Contributions of individual human P450s to the P450 sector of carcinogen activation (Rendic and Guengerich 2012)

more than 100 different genotypes associated with P450 21A2 deficiency, including salt-wasting syndrome (Wedell 2011; Zhao et al. 2012). In addition, deficiencies in the P450s involved in the metabolism of vitamins A and D are the basis of diseases (Nebert and Russell 2002).

**Drug Metabolism** Before understanding of human P450s developed, prediction of human pharmacokinetic behavior of a drug candidate was very difficult, and human pharmacokinetic problems were a major reason for failure of drug candidates in clinical trials (Kola and Landis 2004).

P450s are involved in about 75 % of the enzymatic reactions involved in drug metabolism (Fig. 2.2) (Wienkers and Heath 2005; Williams et al. 2004), and today it is possible to determine which P450s are involved in the metabolism of a new drug candidate using in vitro approaches (Guengerich and Shimada 1991). There are reasonably good approaches to extrapolating to in vivo situations and predicting variability in human populations (Ito et al. 1998; Guest et al. 2011). In addition, both induction and inhibition can be studied in vitro. Other knowledge of the inducers and inhibitors of individual P450s (Guengerich 2005) enables prediction of potential drug–drug interactions (Andersson et al. 2005).

Knowledge about individual P450s involved in reactions and genetic polymorphisms (vide supra) is also used to guide drug prescriptions and use, and this is a major element in “personalized medicine.” As an example, the maintenance levels of the anticoagulant warfarin are related to polymorphism in the *CYP2C9* gene (P450 2C9) (Daly et al. 2002; Garcia and Hylek 2009). Several side effects of drugs (e.g., debrisoquine, perhexiline) are related to P450 2D6 (Idle et al. 1978; Idle and Smith 1979; Oates et al. 1981; Shah et al. 1982). Serious adverse reactions of terfenadine are related to the inhibition of its metabolism by P450 3A4 (and the resulting increased plasma and tissue levels) (Yun et al. 1993; Thompson and Oster 1996; Guengerich 2013, 2014). Induction of P450 3A4 by rifampicin, barbiturates, and herbal medicines containing hyperforin increases the metabolism of the oral



contraceptive 17 $\alpha$ -ethynylestradiol, which can lead to unexpected breakthrough bleeding and pregnancy (Bolt et al. 1975; Guengerich 1988a).

Knowledge of P450 oxidation of drug candidates, induction, and inhibition is widely used today in the overall process of drug development (Humphreys 2008).

**P450s as Drug Targets** Another aspect of P450s in medical practice is their undesired effects and targeting by drugs. One example is the steroid aromatase, P450 19A1, which converts androgens to estrogens (Brodie 1985); this is an issue in estrogen-stimulated tumors. Another target is P450 17A1, which forms androgens and is a target in prostate cancer (DeVore and Scott 2012). Several fungal conditions are treated with antimycotic inhibitors of fungal and yeast P450 51A1 (Aoyama et al. 1998). In this regard, some P450s of *Mycobacterium tuberculosis* have been shown to be required for viability or virulence, and efforts to develop drugs are in progress (Seward et al. 2006; Johnston et al. 2010).

## 2.3 What Have We Left to Learn About P450s: Basic Questions

Another author might provide a different list, but the following is my own opinion. More practical questions about P450s follow later. This selection is biased in part on my own research interests, although we are not working on all aspects.

### 2.3.1 What Are the Functions of the Orphan P450s?

P450s can be classified on the basis of their substrates (Table 2.1). Almost one-fourth of the human P450s are grouped as “orphans” (Guengerich 2005), a term adopted from the orphan steroid nuclear receptor family (Mangelsdorf and Evans 1995). Our laboratory has been involved in systematic searches for functions of these, and some of the progress has been reviewed (Guengerich and Cheng 2011). Recent (and unexpected) results include the oxidations of lysphospholipids by P450 2W1 (Xiao and Guengerich 2012) and of *N*-arachidonoylserotonin by P450 2U1 (Siller et al. 2014). Some drug substrates have been identified for several of the orphans (Nishida et al. 2010; Xiao et al. 2011; Wang and Guengerich 2012; Edson et al. 2013), but there is little current information about the overall contribution of these. Only one of the orphans (2W1; Table 2.1) has been found to activate carcinogens (Wu et al. 2006).

At the present time only speculation is possible as to whether any of these orphan P450s will be shown to have important physiological roles. P450 4F11 hydroxylates vitamin K (Edson et al. 2013). *Cyp2s1*( $-/-$ ) mice are phenotypically normal (X. Ding, personal communication).

When can a P450 be considered to be “deorphanized?” P450 2R1 was, when an important role in vitamin A metabolism was defined (Cheng et al. 2003). However, in another sense, all the P450s under the “Xenobiotics” heading in Table 2.1 can be considered orphans in the sense that no critical physiological reactions have been identified. Elimination of the apparent orthologues in mice has, in most cases, no major observable phenotype in mice, and humans missing some of these are known but are normal unless exposed to certain drugs.

The deorphanization of the myriad of bacterial, insect, and plant P450s is difficult but has important implications in agriculture, pest control, and other practical issues. In many cases there are advantages (compared to mammals and humans) in that gene knockouts can be done and phenotypes can be observed; for example, two P450 genes in *Streptomyces coelicolor* are functional in sporulation (Cheng et al. 2010; Tian et al. 2013), although the reactions underlying these phenomena have not been defined. Exactly how defects in sterol metabolism relate to *M. tuberculosis* is not yet clear (Seward et al. 2006; Johnston et al. 2010). Elucidation of the functions of the function of P450 genes in crop plants, weeds, insects, and fungi has great potential in agriculture (Kinney 2006).

### 2.3.2 *Is There More to Learn About the Nature of Oxidizing Species of P450s?*

As mentioned earlier, all the early proposals about the nature of oxidizing species have now largely culminated with two entities,  $\text{FeO}^{3+}$  (“Compound I”) and  $\text{FeO}_2^-$ , its precursor (Ortiz de Montellano and De Voss 2005). The latter has been used to rationalize some unusual reactions, mainly those with aldehyde substrates (Akhtar et al. 1982). Strong evidence for the role of  $\text{FeO}^{3+}$  has come from the detailed characterization of this entity by Prof. Michael Green and his associates (Rittle and Green 2010). The major evidence for the  $\text{FeO}_2^-$  mechanism comes from (1) site-directed mutagenesis work, mainly with active site Thr mutants (Vaz et al. 1996), and (2)  $^{18}\text{O}$  labeling experiments with some steroids (Akhtar et al. 1982, 1994).

With this as a background, what are the remaining issues? One need is the extension of the studies on the characterized Compound I ( $\text{FeO}^{3+}$ ) to more P450s, thus testing hypotheses about alternate forms. Further, proposals about the spin-state duality of  $\text{FeO}^{3+}$  have been made by Prof. Sasson Shaik on the basis of theoretical considerations (Harris et al. 2000; Shaik et al. 2005), but these have not been experimentally tested. Access to defined Compound I ( $\text{FeO}^{3+}$ ) P450 species should allow these hypotheses to be addressed.

The role of  $\text{FeO}_2^-$  species has been considered, but the duality of alternate reactions ( $\text{FeO}^{3+}$  and  $\text{FeO}_2^-$ ) acting together has not. In some cases both mechanisms have been proposed, such as with P450 19A1 (Hackett et al. 2005; Sen and Hackett 2012). Site-directed mutagenesis approaches cannot be used in a

quantitative approach, but  $^{18}\text{O}$  (and other labeling approaches?) can be. These experiments are now possible, and our preliminary  $^{18}\text{O}_2$  work with P450 19A1 indicates that the  $\text{FeO}^{3+}$  species is involved in the third oxygenation step in the reaction sequence (Yoshimoto and Guengerich, in preparation).

### 2.3.3 *Allosteric Systems and Two Ligands*

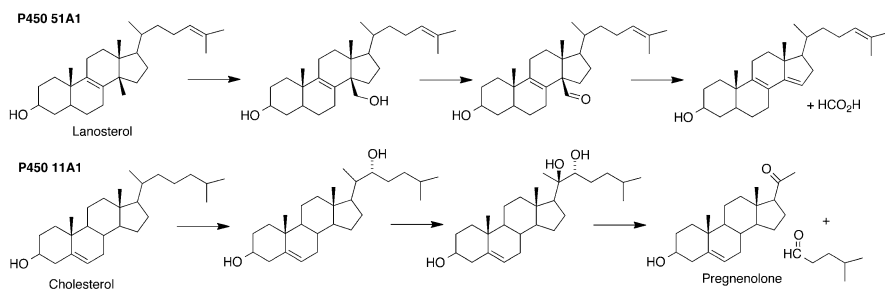
Nonhyperbolic kinetics (homotropic cooperativity) and direct P450 stimulation (heterotropic cooperativity) were first reported 20 years ago (Guengerich et al. 1994; Shou et al. 1994). Since then, these phenomena have been studied extensively, and several indirect lines of evidence led to the proposal that these phenomena could be explained by a model with two (or more) ligands (Shou et al. 1994; Hosea et al. 2000; Davydov et al. 2005; Sligar and Denisov 2007). For many years, however, there was no direct physical proof of this, such as isothermal calorimetry. However, a number of X-ray crystal structures of P450s have now been reported with two ligands in the active site (Zhao et al. 2005, 2012; Ekroos and Sjögren 2006; Schoch et al. 2008). In no case have two *different* ligands both been reported together, and the likelihood of success in such an endeavor is probably very low.

It should be emphasized that not all reports of homotropic cooperativity are necessarily valid. The data for low substrate concentrations, which drive Hill  $n$  values, etc., are especially sensitive to substrate depletion in reactions. Low  $n$  values and excessive deconvolution are suspect.

One of the outstanding issues is explanation and prediction of cooperativity. In particular, cooperativity has not been reported for some of the P450s for which dual occupancy has been observed (Schoch et al. 2008; Zhao et al. 2012). This lack leaves several questions: can we invoke multiple occupancy as a basis for cooperativity if it is not observed? If multiple occupancy is the basis of cooperativity, should it not be observed when the X-ray structures clearly indicate multiple occupancy? Can we predict when we should expect cooperativity? Can the available crystal structures help in this regard?

### 2.3.4 *Do We Really Understand Cytochrome $b_5$ Effects?*

What is clear is that cytochrome  $b_5$  can stimulate a number of P450 catalytic activities. Although multiple explanations have been proposed (Schenkman and Jansson 2003), most of the explanations fit into two hypotheses: (1) providing the “second” electron (step 4) in the general catalytic cycle (Fig. 2.1) and (2) a general allosteric effect that promotes catalytic efficiency. Either effect may reduce the extent of abortive oxygen loss in the cycle. The distinction between the two proposed mechanisms is usually provided by experiments in which the effects of



**Fig. 2.4** Two multi-step human P450 reactions

holo-cytochrome  $b_5$  and apo-cytochrome  $b_5$  (devoid of heme) are compared. Among the human P450 enzymes that are stimulated by cytochrome  $b_5$ , P450s 2A6 (Yun et al. 2005), 2E1, and 4A11 appear to involve electron transfer [i.e., apo-cytochrome is not effective, but P450s 2B6, 2C8, 2C9, 2C19, 3A4, 3A5, and 17A1 (Auchus et al. 1998) do not involve electron transport (Yamazaki et al. 2002)]. [The proposal that the apo-cytochrome  $b_5$  results can be explained by heme transfer from P450s (Guryev et al. 2001) has been dismissed (Yamazaki et al. 2001).]

Biophysical studies with several P450s have provided evidence that the anionic cytochrome  $b_5$  protein is bound to a positively charged region of P450s (Bridges et al. 1998; Estrada et al. 2013). One issue is that this is the same region of P450s proposed to bind NADPH-P450 reductase. Thus, the reductase and cytochrome  $b_5$  would have to switch positions in each cycle of catalysis (Fig. 2.1). This proposal is problematic in a P450 such as 17A1, where cytochrome  $b_5$  only stimulates the second (“lyase”) reaction of a two-step sequence, thus influencing the product distribution (Estrada et al. 2013). Further, the reaction is effectively stimulated by apo-cytochrome  $b_5$ , arguing against a requirement for electron transfer (Auchus et al. 1998). Thus, if cytochrome  $b_5$  occupies the same site as NADPH-P450 reductase (Estrada et al. 2013), then the reductase must be attached to P450 17A1 from steps 2–4 of the catalytic cycle (Fig. 2.1) and then be released for cytochrome  $b_5$  to bind in steps 5–8. Given the instability of the  $\text{FeO}_2^-$  species, this scenario does not seem intuitive but would be consistent with the observation (Estrada et al. 2013). Further investigation of this phenomenon is still in order, even more than 40 years since the first reports of the cytochrome  $b_5$  effects (Correia and Mannering 1973; Hildebrandt and Estabrook 1971).

### 2.3.5 Why Are Some Multi-Step P450 Reactions Processive?

At least five physiological P450 reactions involve multiple steps (Fig. 2.4) (Guengerich et al. 2011). Moreover, multiple oxidations of drugs are commonly observed (Ortiz de Montellano and De Voss 2005; Isin and Guengerich 2007). Although one might view processive reactions (i.e., no equilibration of the

intermediate products with the medium) as being more efficient, such a phenomenon would make specifically inhibiting one step of a multi-step reaction impossible, for example, the so-called lyase reaction of P450 17A1 (DeVore and Scott 2012). Indeed, drugs have been developed to inhibit only this lyase reaction of P450 17A1 (Hara et al. 2013). We have shown the P450 19A1-catalyzed three-step conversion of the androgen androstenedione to estrone is very distributive (the opposite of processive), with each of the intermediates being released from the enzyme and then bound again. Studies on the processivity of the other multi-step P450 steroid oxidations are in progress.

However, some multi-step P450 reactions are processive, such as oxidations of nitrosamines to carboxylic acids by P450s 2A6 and 2E1 (Chowdhury et al. 2010, 2012). The P450 2E1 oxidation of ethanol to acetic acid is also processive (Bell-Parikh and Guengerich 1999), and Prof. Kurt Kunze and his associates have shown that some amine oxidations are processive (Hanson et al. 2010). These are not physiological substrates, and the question is why these are processive. Many of these processive P450 reactions involve carbonyl intermediates, although there does not appear to be any obvious P450 affinity for them (Guengerich et al. 2011). More information is needed in this area.

## 2.4 What Have We Left to Learn About the Practical Issues of P450s?

This list is also not intended to be comprehensive, and it may well be that totally new fields will develop that utilize P450s. This view results from the ability of P450s to catalyze oxidation of so many substrates. In a recent conversation I had with Professor Minor J. Coon, he quipped that if organic chemicals are ever found on another planet, they will probably be substrates for at least one of the (terrestrial) P450s.

### 2.4.1 *Can We Use our Knowledge of P450 Structures Productively?*

The answer is already “yes,” but the real question is how well we can do this in a prospective manner. We now have structures of at least 20 different human P450s (Table 2.1), including all the major “drug-metabolizing” P450s. Most P450s have shown extensive changes upon binding ligands, and therefore ligand-free P450 crystal structures are limited in their usefulness. The malleability of P450 structures is a problem in that different ligands can yield different protein structures for a single P450, for example, P450 3A4 (Ekroos and Sjögren 2006) (rabbit) and P450 2B4 (Shah et al. 2013). Thus, even having a structure of a P450–substrate complex does not necessarily predict the structure of that P450 with a new substrate or ligand.

In practice, drug discovery and development operate on what is sometimes called a “2-week” scale, when new leads need to be reevaluated in a short time. The time needed to obtain crystals and solve a structure is still longer than two weeks and therefore not compatible with industry needs. The hope is that as scientists collect more P450 structures, each P450 will have a finite but limited number of major conformations and that there can be used practically with new substrates. Obviously, this is a very important area for practical research. My own opinion is that actual experiments will still need to be done after virtual screening. The prediction of rates of oxidation is much more difficult than (qualitative) prediction of sites of oxidation in a molecule.

#### ***2.4.2 Can We Relate P450 Polymorphisms to Chronic Diseases?***

As mentioned earlier, knowledge about human P450s has been very useful in advancing drug development and even clinical practice. Polymorphisms can have dramatic influences on drug metabolism. As also pointed out earlier, deficiencies in the steroid-metabolizing P450s have major consequences.

However, what is not yet very clear is how variations in P450s affect chronic diseases. There are several issues here. One is cancer. P450s were studied extensively in part because of chemical carcinogenesis (Conney 1982; Guengerich 1988a, b), and two thirds of the bioactivation reactions with carcinogens are catalyzed by P450s (one half of which are family 1 P450s) (Fig. 2.3) (Rendic and Guengerich 2012). Changes in P450s in animal models can have dramatic effects on cancer incidence (Guengerich 1988b; Gonzalez 2004), but to date relationships in humans have not been so clear. The early relationship of inducibility of aryl hydrocarbon hydroxylation activation with lung cancer (Kellerman et al. 1973a, b) is probably best understood in the context of P450 1B1 (Toide et al. 2003), although this field does not seem to have been addressed again recently. Despite early excitement (Ayesh et al. 1984), the association of decreased lung cancer with the P450 2D6 poor metabolizer phenotype could never be validated (Rostami-Hodjegan et al. 1998). One of the issues in this field is that epidemiology proceeds in the absence of basic science. There is a weak association of P450 1A2 with colon cancer, but only if *N*-acetyltransferase status and consumption of well-done meat are factored in (Lang et al. 1994). Another possibility is P450 2A6 and tobacco-related cancer, which is complicated in that individuals with a deficiency may smoke less because they lack the ability to clear nicotine (Swan et al. 2005). Other epidemiological association of cancers with P450 polymorphisms have either been weak or not confirmed in more extensive studies (d’Errico et al. 1996; Tamaki et al. 2011).

Other efforts have shown weak associations between P450 2D6 status and Parkinson’s disease (Halling et al. 2008).

An interesting relationship has been reported for a polymorphism in P450 4A11 (rs1126742) and hypertension (Gainer et al. 2005). The basis is still unclear. P450 4A11 converts arachidonic acid to its 20-hydroxy product (20-HETE), and the polymorphic variant (coding for a P434S mutation) has been reported to have 60 % of the catalytic efficiency of the wild-type protein (Gainer et al. 2005). However, *Cyp4a10*( $-/-$ ) mice show hypertension but do not produce 20-HETE (Nakagawa et al. 2006), and a transgenic mouse that expresses the human 20-hydroxylase, P450 4A11 (Savas et al. 2009), is also hypertensive (E.F. Johnson, personal communication). In mouse models, deletion of *Cyp2c* subfamily genes can also make animals hypersensitive (Sun et al. 2012). Other work with animals has led to postulates of roles of P450 4F and 2J enzymes in hypertension and other cardiovascular effects (Deng et al. 2011; Yang et al. 2001).

These examples show the complexity of the association. Clearly, more questions remain to be addressed.

### 2.4.3 Application of P450s in Toxicology and Other Assays

Another challenge regarding P450s is their effective application in toxicology. Many successes have already been realized with transgenic mouse models, for example, roles of individual P450s in the toxicity of certain chemicals (Lee et al. 1996). These approaches will continue to develop, particularly as key enzyme and receptor systems are “humanized,” that is, mouse systems are replaced (in the mice) by the human counterparts. The success of this approach has already been demonstrated in mice expressing the human AhR (aryl hydrocarbon receptor) and PPAR $\alpha$  (peroxisomal proliferating activator receptor- $\alpha$ ) receptors (Moriguchi et al. 2003; Yang et al. 2008). Another developing area with transgenic mice may be in response to the need to characterize “human-specific” metabolites (i.e., those constituting  $\geq 10$  % of metabolites not found in animals) for safety testing (Guengerich 2006).

Another important need is in high-throughput toxicology assays. The importance of metabolic capability is generally appreciated in the pharmaceutical industry, given the history (Kola and Landis 2004), and the emphasis on adsorption/distribution/metabolism/excretion work in drug development. However, this is not necessarily the case with other chemicals. A battery of in vitro toxicity tests is being applied to a very large number of compounds in the Toxcast testing program supported by several agencies of the United States government (Sipes et al. 2013), but in the absence of metabolism systems. Although this approach may yield information about the intrinsic toxicities of individual chemicals, the predictive value of the exercise may be limited, just as was the relationship between mutagens and carcinogens before the incorporation of liver extracts with the Ames test (Ames et al. 1973). Aflatoxin B<sub>1</sub> would not be considered very toxic without bioactivation, and the mutagenicity of dinitropyrenes would be overestimated. Exactly which systems should be used needs to be decided for large-scale, low-volume screens. Individual P450 systems are probably not the most appropriate for broad screening, and a multi-P450 system

(including P450s known to oxidize most of the known drugs and carcinogens) (Rendic and Guengerich 2012; Wienkers and Heath 2005; Williams et al. 2004) should be considered, coexpressed with NADPH-P450 reductase. This is one possibility, and others might be better, but this topic deserves attention.

#### ***2.4.4 Applications with Plants and Agriculture***

The final area for consideration is agriculture. As mentioned earlier, plant genomes contain hundreds of P450s. We know little about which of these might be significant in the production of important crops, such as rice, corn (maize), and soybeans. State-of-the-art genomic approaches can now be applied, such as genome-wide association studies (GWAS) for traits and analysis of phenotypic function with gene knockout technology. Using such approaches, we may well find that certain P450s are beneficial (and can be overexpressed) and that others are detrimental to desired properties (and could be targets for development of inhibitory chemicals).

The approaches are designed to increase crop production. Another important aspect is control of pests, including weeds, insects, and fungi. These genomes are being developed rapidly, as are similar approaches (GWAS, knockouts) to find targets for inhibition of function. As an example, one could develop a herbicide from an inhibitor of a key P450 in a weed. Another approach is to overexpress a herbicide-metabolizing P450 gene into a cereal crop (e.g., rice or corn). These approaches are very feasible and can be used without the ethical issues inherent in human medicine. I realize that there is political resistance to biotechnology in Europe, but the need to feed the 7 billion people in the world should trump such concerns, given the very safe record of genomic agriculture today.

The last point under agricultural application of P450s involves animals and veterinary issues. P450 applications in human medicine have developed rapidly, but the same concerns about drug–drug interactions, polymorphisms, etc. certainly apply in veterinary pharmacology. I recently reviewed a grant application (from another country) and learned that the P450 repertoire in horses is largely unknown, beyond the genomic level, and the prescription of drugs cannot be guided by such information. I am sure that we also need to know more about P450s in important domestic animals such as cattle, swine, and sheep (as well as sport and companion animals, e.g., horses, dogs, cats) to treat them more effectively.

### **2.5 Epilogue**

The P450 world has been exciting for many of us. We have already seen tremendous advancement at the basic level, and experiments we could only dream of 40 years ago are routine today. The number of papers published on P450s continues to grow (<http://webtools.mf.uni-lj.si/public/medsum.html>). As the field has



matured, the bar for publishing P450 papers continues to be set higher. The success of P450 research in medicine has been remarkable, more than first imagined. The application of P450 research continues to grow in many fields, and we will undoubtedly see more of this progress.

Finally, it remains for me to thank two of our senior members of the P450 field, Professors Tsuneo Omura and Minor J. Coon, for their discoveries and contributions to the field, which are very much appreciated.

**Acknowledgments** I thank Kathleen Trisler for her assistance in preparation of the manuscript. P450 research in this laboratory is currently supported by National Institutes of Health grants R37 CA090426, P01 DK038226, and R01 GM103937.

Finally, this chapter is dedicated to the memory of two of the pioneers in this field, Professors Ronald W. Estabrook and Allan H. Conney. Both died in 2013 (August and September, respectively). Professor Estabrook was involved in critical experiments that established P450 as the terminal oxidase in the microsomal electron transport chain. Professor Conney was involved in the discovery of P450 induction as a graduate student with Professors James and Elizabeth Miller, made important contributions regarding the multiplicity of P450s, and, together with Dr. Donald Jerina, established the bay-region diol epoxide pathway for activation of polycyclic aromatic hydrocarbons and its significance in chemical carcinogenesis.

## References

- Addya S, Anandatheerthavarada HK, Biswas G, Bhagwat SV, Mullick J, Avadhani NG (1997) Targeting of NH<sub>2</sub>-terminal-processed microsomal protein to mitochondria: a novel pathway for the biogenesis of hepatic mitochondrial P450<sub>mt2</sub>. *J Cell Biol* 139:589–599
- Akhtar M, Calder MR, Corina DL, Wright JN (1982) Mechanistic studies on C-19 demethylation in oestrogen biosynthesis. *Biochem J* 201:569–580
- Akhtar M, Corina D, Miller S, Shyadehi AZ, Wright JN (1994) Mechanism of the acyl-carbon cleavage and related reactions catalyzed by multifunctional P-450s: studies on cytochrome P450<sub>17α</sub>. *Biochemistry* 33:4410–4418
- Alvares AP, Schilling G, Levin W, Kuntzman R (1967) Studies on the induction of CO-binding pigments in liver microsomes by phenobarbital and 3-methylcholanthrene. *Biochem Biophys Res Commun* 29:521–526
- Ames BN, Durston WE, Yamasaki E, Lee FD (1973) Carcinogens are mutagens: a simple test system combining liver homogenates for activation and bacteria for detection. *Proc Natl Acad Sci USA* 70:2281–2285
- Andersson T, Flockhart DA, Goldstein DB, Huang SM, Kroetz DL, Milos PM, Ratain MJ, Thummel K (2005) Drug-metabolizing enzymes: evidence for clinical utility of pharmacogenomic tests. *Clin Pharmacol Ther* 78:559–581
- Aoyama Y, Horiuchi T, Gotoh O, Noshiro M, Yoshida Y (1998) *Cyp51*-like gene of *Mycobacterium tuberculosis* actually encodes a P450 similar to eukaryotic CYP51. *J Biochem (Tokyo)* 124:694–696
- Auchus RJ, Lee TC, Miller WL (1998) Cytochrome *b<sub>5</sub>* augments the 17,20-lyase activity of human P450c17 without direct electron transfer. *J Biol Chem* 273:3158–3165
- Ayesh R, Idle JR, Ritchie JC, Crothers MJ, Hetzel MR (1984) Metabolic oxidation phenotypes as markers for susceptibility to lung cancer. *Nature (Lond)* 312:169–170
- Bajpai P, Sangar MC, Tang W, Chowdhury G, Cheng Q, Fang J-K, Martin MV, Guengerich FP, Avadhani NG (2013) Metabolism of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine by

- mitochondria-targeted cytochrome P450 2D6: implications for Parkinson's disease. *J Biol Chem* 288:4436–4451
- Bansal S, Liu C-P, Sepuri NBV, Anandatheerthavarada HK, Guengerich FP, Avadhani NG (2010) Mitochondria-targeted cytochrome P450 2E1 preferentially induces oxidative damage and augments alcohol mediated mitochondrial dysfunction in cultured cells. *J Biol Chem* 285:24609–24619
- Beaune P, Kremers PG, Kaminsky LS, de Graeve J, Guengerich FP (1986) Comparison of monooxygenase activities and cytochrome P-450 isozyme concentrations in human liver microsomes. *Drug Metab Dispos* 14:437–442
- Bell-Parikh LC, Guengerich FP (1999) Kinetics of cytochrome P450 2E1-catalyzed oxidation of ethanol to acetic acid via acetaldehyde. *J Biol Chem* 274:23833–23840
- Bolt HM, Kappus H, Bolt M (1975) Effect of rifampicin treatment on the metabolism of oestradiol and 17 $\alpha$ -ethinyloestradiol by human liver microsomes. *Eur J Clin Pharmacol* 8:301–307
- Bridges A, Gruenke L, Chang Y-T, Vakser IA, Loew G, Waskell L (1998) Identification of the binding site on cytochrome P450 2B4 for cytochrome *b*<sub>5</sub> and cytochrome P450 reductase. *J Biol Chem* 273:17036–17049
- Brodie AMH (1985) Aromatase inhibition and its pharmacologic implications. *Biochem Pharmacol* 34:3213–3219
- Cheng JB, Motola DL, Mangelsdorf DJ, Russell DW (2003) De-orphanization of cytochrome P450 2R1: a microsomal vitamin D 25-hydroxylase. *J Biol Chem* 278:38084–38093
- Cheng Q, Lamb DC, Kelly SL, Li L, Guengerich FP (2010) Cyclization of a cellular dipentaenone by *Streptomyces coelicolor* cytochrome P450 154A1 without oxidation reduction. *J Am Chem Soc* 132:15173–15175
- Chowdhury G, Calcutt MW, Guengerich FP (2010) Oxidation of *N*-nitrosodimethylamine and *N*-nitrosodiethylamine by human cytochrome P450 2A6: sequential oxidation to carboxylic acids and analysis of reaction steps. *J Biol Chem* 285:8031–8044
- Chowdhury G, Calcutt MW, Nagy LD, Guengerich FP (2012) Oxidation of methyl and ethyl nitrosamines by cytochromes P450 2E1 and 2B1. *Biochemistry* 51:9995–10007
- Coelho PS, Brustad EM, Kannan A, Arnold FH (2013) Olefin cyclopropanation via carbene transfer catalyzed by engineered cytochrome P450 enzymes. *Science* 339:307–310
- Conney AH (1982) Induction of microsomal enzymes by foreign chemicals and carcinogenesis by polycyclic aromatic hydrocarbons: G. H. A. Clowes Memorial Lecture. *Cancer Res* 42:4875–4917
- Correia MA, Mannering GJ (1973) Reduced diphosphopyridine nucleotide synergism of the reduced triphosphopyridine nucleotide-dependent mixed-function oxidase system of hepatic microsomes. II. Role of the type I drug-binding site of cytochrome P-450. *Mol Pharmacol* 9:470–485
- d'Errico A, Taioli E, Chen X, Vineis P (1996) Genetic metabolic polymorphisms and the risk of cancer: a review of the literature. *Biomarkers* 1:149–173
- Daly AK, Day CP, Aithal GP (2002) CYP2C9 polymorphism and warfarin dose requirements. *Br J Clin Pharmacol* 53:408–409
- Davydov DR, Botchkareva AE, Davydova NE, Halpert JR (2005) Resolution of two substrate-binding sites in an engineered cytochrome P450<sub>eryf</sub> bearing a fluorescent probe. *Biophys J* 89:418–432
- Deng Y, Edin ML, Theken KN, Schuck RN, Flake GP, Kannon MA, DeGraff LM, Lih FB, Foley J, Bradbury JA, Graves JP, Tomer KB, Falck JR, Zeldin DC, Lee CR (2011) Endothelial CYP epoxigenase overexpression and soluble epoxide hydrolase disruption attenuate acute vascular inflammatory responses in mice. *FASEB J* 25:703–713
- DeVore NM, Scott EE (2012) Structures of cytochrome P450 17A1 with prostate cancer drugs abiraterone and TOK-001. *Nature (Lond)* 482:116–119
- Diehl H, Schädelin J, Ullrich V (1970) Studies on the kinetics of cytochrome P-450 reduction in rat liver microsomes. *Hoppe Seylers Z Physiol Chem* 351:1359–1371

- Distlerath LM, Reilly PEB, Martin MV, Davis GG, Wilkinson GR, Guengerich FP (1985) Purification and characterization of the human liver cytochromes P-450 involved in debrisoquine 4-hydroxylation and phenacetin *O*-deethylation, two prototypes for genetic polymorphism in oxidative drug metabolism. *J Biol Chem* 260:9057–9067
- Edson K, Prasad B, Unadkat JD, Suhara Y, Okano T, Guengerich FP, Rettie AE (2013) Cytochrome P450 dependent catabolism of vitamin K: initiation of  $\omega$ -hydroxylation of human CYP4F2 and CYP4F11. *Biochemistry* 52:8276–8285
- Ekkroos M, Sjögren T (2006) Structural basis for ligand promiscuity in cytochrome P450 3A4. *Proc Natl Acad Sci USA* 103:13862–13867
- Estabrook RW, Cooper DY, Rosenthal O (1963) The light reversible carbon monoxide inhibition of the steroid C21-hydroxylase system of the adrenal cortex. *Biochem Z* 338:741–755
- Estabrook RW, Franklin MR, Cohen B, Shigamatzu A, Hildebrandt AG (1971) Biochemical and genetic factors influencing drug metabolism. Influence of hepatic microsomal mixed function oxidation reactions on cellular metabolic control. *Metabolism* 20:187–199
- Estrada DF, Laurence JS, Scott EE (2013) Substrate-modulated cytochrome P450 17A1 and cytochrome *b*<sub>5</sub> interactions revealed by NMR. *J Biol Chem* 288:17008–17018
- Evans WE, McLeod HL (2003) Pharmacogenomics—drug disposition, drug targets, and side effects. *N Engl J Med* 348:538–549
- Gainer JV, Bellamine A, Dawson EP, Womble KE, Grant SW, Wang Y, Cupples LA, Guo CY, Demissie S, O'Donnell CJ, Brown NJ, Waterman MR, Capdevila JH (2005) Functional variant of CYP4A11 20-hydroxyecosatetraenoic acid synthase is associated with essential hypertension. *Circulation* 111:63–69
- Garcia DA, Hylek E (2009) Warfarin pharmacogenetics. *N Engl J Med* 360:2474, author reply 2475
- Garfinkel D (1958) Studies on pig liver microsomes. I. Enzymic and pigment composition of different microsomal fractions. *Arch Biochem Biophys* 77:493–509
- Gigon PL, Gram TE, Gillette JR (1969) Studies on the rate of reduction of hepatic microsomal cytochrome P-450 by reduced nicotinamide adenine dinucleotide phosphate: effect of drug substrates. *Mol Pharmacol* 5:109–122
- Gillette JR, Brodie BB, La Du BN (1957) The oxidation of drugs by liver microsomes: on the role of TPNH and oxygen. *J Pharmacol Exp Ther* 119:532–540
- Gomez A, Ingleman-Sundberg M (2009) Epigenetic and microRNA-dependent control of cytochrome P450 expression: a gap between DNA and protein. *Pharmacogenomics* 10:1067–1076
- Gonzalez FJ (2004) Cytochrome P450 humanised mice. *Hum Genomics* 1:300–306
- Gonzalez FJ, Skoda RC, Kimura S, Umeno M, Zanger UM, Nebert DW, Gelboin HV, Hardwick JP, Meyer UA (1988) Characterization of the common genetic defect in humans deficient in debrisoquine metabolism. *Nature (Lond)* 331:442–446
- Gorsky LD, Koop DR, Coon MJ (1984) On the stoichiometry of the oxidase and monooxygenase reactions catalyzed by liver microsomal cytochrome P-450: products of oxygen reduction. *J Biol Chem* 259:6812–6817
- Guengerich FP (1988a) Oxidation of 17 $\alpha$ -ethynylestradiol by human liver cytochrome P-450. *Mol Pharmacol* 33:500–508
- Guengerich FP (1988b) Roles of cytochrome P-450 enzymes in chemical carcinogenesis and cancer chemotherapy. *Cancer Res* 48:2946–2954
- Guengerich FP (2001) Common and uncommon cytochrome P450 reactions related to metabolism and chemical toxicity. *Chem Res Toxicol* 14:611–650
- Guengerich FP (2002) Cytochrome P450 enzymes in the generation of commercial products. *Nat Rev Drug Discov* 1:359–366
- Guengerich FP (2005) Human cytochrome P450 enzymes. In: Ortiz de Montellano PR (ed) *Cytochrome P450: structure, mechanism, and biochemistry*, 3rd edn. Kluwer Academic/Plenum Press, New York, pp 377–530
- Guengerich FP (2006) Safety assessment of stable drug metabolites. *Chem Res Toxicol* 19:1559–1560

- Guengerich FP (2013) Kinetic deuterium isotope effects in cytochrome P450 reactions. *J Labelled Comp Radiopharm* 56:428–431
- Guengerich FP (2014) Cytochrome P450-mediated drug interactions and cardiovascular toxicity: the Seldane to Allegra transformation. In: Wang J, Urban L (eds) *Predictive ADMET: integrated approaches in drug discovery and development*. Wiley, New York, Chap. 23, pp 523–534
- Guengerich FP, Cheng Q (2011) Orphans in the human cytochrome P450 family: approaches to discovering function and relevance to pharmacology. *Pharmacol Rev* 63:684–699
- Guengerich FP, Isin EM (2014) Unusual metabolic reactions and pathways. In: Lee P, Aizawa H, Gau L, Prakash C, Zhong D (eds) *The handbook of metabolic pathways of xenobiotics*. Wiley, Chichester, UK, pp 147–197.
- Guengerich FP, Johnson WW (1997) Kinetics of ferric cytochrome P450 reduction by NADPH-cytochrome P450 reductase: rapid reduction in absence of substrate and variations among cytochrome P450 systems. *Biochemistry* 36:14741–14750
- Guengerich FP, Munro AW (2013) Unusual cytochromes P450: enzymes and reactions. *J Biol Chem* 288:17063–17069
- Guengerich FP, Shimada T (1991) Oxidation of toxic and carcinogenic chemicals by human cytochrome P-450 enzymes. *Chem Res Toxicol* 4:391–407
- Guengerich FP, Kim B-R, Gillam EMJ, Shimada T (1994) Mechanisms of enhancement and inhibition of cytochrome P450 catalytic activity. In: Lechner MC (ed) *Proceedings of the 8th international conference on cytochrome P450: biochemistry, biophysics, and molecular biology*. John Libbey Eurotext, Paris, pp 97–101
- Guengerich FP, Sohl CD, Chowdhury G (2011) Multi-step oxidations catalyzed by cytochrome P450 enzymes: processive vs. distributive kinetics and the issue of carbonyl oxidation. *Arch Biochem Biophys* 507:126–134
- Guest EJ, Rowland-Yeo K, Rostami-Hodjegan A, Tucker GT, Houston JB, Galetin A (2011) Assessment of algorithms for predicting drug–drug interactions via inhibition mechanisms: comparison of dynamic and static models. *Br J Clin Pharmacol* 71:72–87
- Guryev OL, Gilep AA, Usanov SA, Estabrook RW (2001) Interaction of apo-cytochrome *b*<sub>5</sub> with cytochromes P4503A4 and P45017A: relevance of heme transfer reactions. *Biochemistry* 40:5018–5031
- Gut J, Catin T, Dayer P, Kronbach T, Zanger U, Meyer UA (1986) Debrisoquine/sparteine-type polymorphism of drug oxidation: purification and characterization of two functionally different human liver cytochrome P-450 isozymes involved in impaired hydroxylation of the prototype substrate bufuralol. *J Biol Chem* 261:11734–11743
- Hackett JC, Brueggemeier RW, Hadad CM (2005) The final catalytic step of cytochrome P450 aromatase: a density functional theory study. *J Am Chem Soc* 127:5224–5237
- Halling J, Petersen MS, Grandjean P, Weihe P, Brosten K (2008) Genetic predisposition to Parkinson's disease: CYP2D6 and HFE in the Faroe Islands. *Pharmacogenet Genomics* 18:209–212
- Hanson KL, VandenBrink BM, Babu KN, Allen KE, Nelson WL, Kunze KL (2010) Sequential metabolism of secondary alkyl amines to metabolic-intermediate complexes: opposing roles for the secondary hydroxylamine and primary amine metabolites of desipramine, (*S*)-fluoxetine, and *N*-desmethyldiltiazem. *Drug Metab Dispos* 38:963–972
- Hara T, Kouno J, Kaku T, Takeuchi T, Kusaka M, Tasaka A, Yamaoka M (2013) Effect of a novel 17,20-lyase inhibitor, orteronel (TAK-700), on androgen synthesis in male rats. *J Steroid Biochem Mol Biol* 134:80–91
- Harris N, Cohen S, Filatov M, Ogliaro F, Shaik S (2000) Two-state reactivity in the rebound step of alkane hydroxylation by cytochrome P-450: origins of free radicals with finite lifetimes. *Angew Chem Int Ed* 39:2003–2007
- Henderson CJ, McLaughlin LA, Wolf CR (2013) Evidence that cytochrome *b*<sub>5</sub> and cytochrome *b*<sub>5</sub> reductase can act as sole electron donors to the hepatic cytochrome P450 systems. *Mol Pharmacol* 83:1209–1217
- Hildebrandt A, Estabrook RW (1971) Evidence for the participation of cytochrome *b*<sub>5</sub> in hepatic microsomal mixed-function oxidation reactions. *Arch Biochem Biophys* 143:66–79

- Hildebrandt A, Remmer H, Estabrook RW (1968) Cytochrome P-450 of liver microsomes: one pigment or many. *Biochem Biophys Res Commun* 30:607–612
- Hosea NA, Miller GP, Guengerich FP (2000) Elucidation of distinct binding sites for cytochrome P450 3A4. *Biochemistry* 39:5929–5939
- Hughes AL, Powell DW, Bard M, Eckstein J, Barbuch R, Link AJ, Espenshade PJ (2007) DAP1/PGRMC1 binds and regulates cytochrome P450 enzymes. *Cell Metab* 5:143–149
- Humphreys WG (2008) Drug metabolism research as an integral part of the drug discovery process. In: Zhang D, Zhu M, Humphreys WG (eds) *Drug metabolism in drug design and development*. Wiley, Hoboken, Chap. 8, pp 239–260
- Idle JR, Smith RL (1979) Polymorphisms of oxidation at carbon centers of drugs and their clinical significance. *Drug Metab Rev* 9:301–317
- Idle JR, Mahgoub A, Lancaster R, Smith RL (1978) Hypotensive response to debrisoquine and hydroxylation phenotype. *Life Sci* 22:979–984
- Isin EM, Guengerich FP (2006) Kinetics and thermodynamics of ligand binding by cytochrome P450 3A4. *J Biol Chem* 281:9127–9136
- Isin EM, Guengerich FP (2007) Complex reactions catalyzed by cytochrome P450 enzymes. *Biochim Biophys Acta* 1770:314–329
- Ito K, Iwatsubo T, Kanamitsu S, Ueda K, Suzuki H, Sugiyama Y (1998) Prediction of pharmacokinetic alterations caused by drug–drug interactions: metabolic interaction in the liver. *Pharmacol Rev* 50:387–411
- Johnston JB, Ouellet H, Ortiz de Montellano PR (2010) Functional redundancy of steroid C26-monooxygenase activity in *Mycobacterium tuberculosis* revealed by biochemical and genetic analyses. *J Biol Chem* 285:36352–36360
- Kalow W (1962) *Pharmacogenetics*. Saunders, Philadelphia
- Katagiri M, Ganguli BN, Gunsalus IC (1968) A soluble cytochrome P450 functional in methylene hydroxylation. *J Biol Chem* 243:3543–3546
- Kellerman G, Luyten-Kellerman M, Shaw CR (1973a) Genetic variation of aryl hydrocarbon hydroxylase in human lymphocytes. *Am J Hum Genet* 25:327–331
- Kellerman G, Shaw CR, Luyten-Kellerman M (1973b) Aryl hydrocarbon hydroxylase inducibility and bronchogenic carcinoma. *N Engl J Med* 298:934–937
- Kinney AJ (2006) Metabolic engineering in plants for human health and nutrition. *Curr Opin Biotechnol* 17:130–138
- Klingenberg M (1958) Pigments of rat liver microsomes. *Arch Biochem Biophys* 75:376–386
- Kola I, Landis J (2004) Can the pharmaceutical industry reduce attrition rates? *Nat Rev Drug Discov* 3:711–715
- Lang NP, Butler MA, Massengill J, Lawson M, Stotts RC, Maurer-Jensen M, Kadlubar FF (1994) Rapid metabolic phenotypes for acetyltransferase and cytochrome P4501A2 and putative exposure to food-borne heterocyclic amines increase the risk for colorectal cancer or polyps. *Cancer Epidemiol Biom* 3:675–682
- Lee SST, Buters JTM, Pineau T, Fernandez-Salguero P, Gonzalez FJ (1996) Role of Cyp2e1 in the hepatotoxicity of acetaminophen. *J Biol Chem* 271:12063–12067
- Lu AYH, Coon MJ (1968) Role of hemoprotein P-450 in fatty acid  $\omega$ -hydroxylation in a soluble enzyme system from liver microsomes. *J Biol Chem* 243:1331–1332
- Mahgoub A, Idle JR, Dring LG, Lancaster R, Smith RL (1977) Polymorphic hydroxylation of debrisoquine in man. *Lancet* 2:584–586
- Mangelsdorf DJ, Evans RM (1995) The RXR heterodimers and orphan receptors. *Cell* 83:841–850
- McLean KJ, Sabri M, Marshall KR, Lawson RJ, Lewis DG, Clift D, Balding PR, Dunford AJ, Warman AJ, McVey JP, Quinn AM, Sutcliffe MJ, Scrutton NS, Munro AW (2005) Biodiversity of cytochrome P450 redox systems. *Biochem Soc Trans* 33:796–801
- Miller WL, Auchus RJ (2011) The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders. *Endocr Rev* 32:81–151
- Mizutani M, Sato F (2011) Unusual P450 reactions in plant secondary metabolism. *Arch Biochem Biophys* 507:194–203

- Moriguchi T, Motohashi H, Hosoya T, Nakajima O, Takahashi S, Ohsako S, Aoki Y, Nishimura N, Tohyama C, Fujii-Kuriyama Y, Yamamoto M (2003) Distinct response to dioxin in an arylhydrocarbon receptor (*Ahr*)-humanized mouse. *Proc Natl Acad Sci USA* 100:5652–5657
- Mueller GC, Miller JA (1948) The metabolism of 4-dimethylaminoazobenzene by rat liver homogenates. *J Biol Chem* 176:535–544
- Mueller GC, Miller JA (1953) The metabolism of methylated aminoazo dyes. II. Oxidative demethylation by rat liver homogenates. *J Biol Chem* 202:579–587
- Mueller EJ, Loida PJ, Sligar SG (1995) Twenty-five years of P450<sub>cam</sub> research: mechanistic insights into oxygenase catalysis. In: Ortiz de Montellano PR (ed) *Cytochrome P450: structure, mechanism, and biochemistry*, 2nd edn. Plenum, New York, Chap. 3, pp 83–124
- Mutoh S, Sobhany M, Moore R, Perera L, Pedersen L, Sueyoshi T, Negishi M (2013) Phenobarbital indirectly activates the constitutive active androstane receptor (CAR) by inhibition of epidermal growth factor receptor signaling. *Sci Signal* 6:ra31
- Nakagawa K, Holla VR, Wei Y, Wang WH, Gatica A, Wei S, Mei S, Miller CM, Cha DR, Price E Jr, Zent R, Pozzi A, Breyer MD, Guan Y, Falck JR, Waterman MR, Capdevila JH (2006) Salt-sensitive hypertension is associated with dysfunctional *Cyp4a10* gene and kidney epithelial sodium channel. *J Clin Invest* 116:1696–1702
- Nebert DW, Russell DW (2002) Clinical importance of the cytochromes P450. *Lancet* 360:1155–1162
- Nelson DO, Lorusso DJ, Mannering GJ (1973) Requirement of a soluble protein for maximal activity of the monooxidase system of hepatic microsomes. *Biochem Biophys Res Commun* 53:995–1001
- Niranjan BG, Avadhani NG (1980) Activation of aflatoxin B<sub>1</sub> by a monooxygenase system localized in rat liver mitochondria. *J Biol Chem* 255:6575–6578
- Nishida CR, Lee M, Ortiz de Montellano PR (2010) Efficient hypoxic activation of the anticancer agent AQ4N by CYP2S1 and CYP2W1. *Mol Pharmacol* 78:497–502
- Oates NS, Shah RR, Idle JR, Smith RL (1981) Phenformin-induced lactic acidosis associated with impaired debrisoquine hydroxylation. *Lancet* 1:837–838
- Omura T, Sato R (1962) A new cytochrome in liver microsomes. *J Biol Chem* 237:1375–1376
- Omura T, Sato R (1964a) The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. *J Biol Chem* 239:2370–2378
- Omura T, Sato R (1964b) The carbon monoxide-binding pigment of liver microsomes. II. Solubilization, purification, and properties. *J Biol Chem* 239:2379–2385
- Ortiz de Montellano PR, De Voss JJ (2005) Substrate oxidation by cytochrome P450 enzymes. In: Ortiz de Montellano PR (ed) *Cytochrome P450: structure, mechanism, and biochemistry*, 3rd edn. Kluwer Academic/Plenum Press, New York, pp 183–245
- Ortiz de Montellano PR, DeVoss JJ (2002) Oxidizing species in the mechanism of cytochrome P450. *Nat Prod Rep* 19:477–493
- Peterson JA, Ebel RE, O’Keeffe DH, Matsubara T, Estabrook RW (1976) Temperature dependence of cytochrome P-450 reduction. A model for NADPH-cytochrome P-450 reductase: cytochrome P-450 interaction. *J Biol Chem* 251:4010–4016
- Plum LA, DeLuca HF (2010) Vitamin D, disease and therapeutic opportunities. *Nat Rev Drug Discov* 9:941–955
- Poulos TL, Finzel BC, Gunsalus IC, Wagner GC, Kraut J (1985) The 2.6-Å crystal structure of *Pseudomonas putida* cytochrome P-450. *J Biol Chem* 260:16122–16130
- Rendic S, Guengerich FP (2012) Contributions of human enzymes in carcinogen metabolism. *Chem Res Toxicol* 25:1316–1383
- Rittle J, Green MT (2010) Cytochrome P450 compound I: capture, characterization, and C–H bond activation kinetics. *Science* 330:933–937
- Rostami-Hodjegan A, Lennard MS, Woods HF, Tucker GT (1998) Meta-analysis of studies of the CYP2D6 polymorphism in relation to lung cancer and Parkinson’s disease. *Pharmacogenetics* 8:227–238

- Ryan KJ (1958) Conversion of androstenedione to estrone by placental microsomes. *Biochim Biophys Acta* 27:658–662
- Savas U, Machemer DE, Hsu MH, Gaynor P, Lasker JM, Tukey RH, Johnson EF (2009) Opposing roles of peroxisome proliferator-activated receptor alpha and growth hormone in the regulation of *CYP4A11* expression in a transgenic mouse model. *J Biol Chem* 284:16541–16552
- Schenkman JB, Jansson I (2003) The many roles of cytochrome *b<sub>5</sub>*. *Pharmacol Ther* 97:139–152
- Schoch GA, Yano JK, Sansen S, Dansette PM, Stout CD, Johnson EF (2008) Determinants of cytochrome P450 2C8 substrate binding: structures of complexes with montelukast, troglitazone, felodipine, and 9-*cis*-retinoic acid. *J Biol Chem* 283:17227–17237
- Sen K, Hackett JC (2012) Coupled electron transfer and proton hopping in the final step of CYP19-catalyzed androgen aromatization. *Biochemistry* 51:3039–3049
- Sevrioukova IF, Poulos TL (2012) Structural and mechanistic insights into the interaction of cytochrome P450 3A4 with bromoergocryptine, a type I ligand. *J Biol Chem* 287:3510–3517
- Seward HE, Roujeinikova A, McLean KJ, Munro AW, Leys D (2006) Crystal structure of the *Mycobacterium tuberculosis* P450 CYP121-fluconazole complex reveals new azole drug-P450 binding mode. *J Biol Chem* 281:39437–39443
- Shah RR, Oates NS, Idle JR, Smith RL, Lockhart JDF (1982) Impaired oxidation of debrisoquine in patients with perhexiline neuropathy. *Br Med J* 284:295–299
- Shah MB, Kufareva I, Pascual J, Zhang QH, Stout CD, Halpert JR (2013) A structural snapshot of CYP2B4 in complex with paroxetine provides insights into ligand binding and clusters of conformational states. *J Pharmacol Exp Ther* 346:113–120
- Shaik S, Kumar D, de Visser SP, Altun A, Thiel W (2005) Theoretical perspective on the structure and mechanism of cytochrome P450 enzymes. *Chem Rev* 105:2279–2328
- Shiro Y, Fujii M, Iizuka T, Adachi S, Tsukamoto K, Nakahara K, Shoun H (1995) Spectroscopic and kinetic studies on reaction of cytochrome P450<sub>nor</sub> with nitric oxide: implication for its nitric oxide reduction mechanism. *J Biol Chem* 270:1617–1623
- Shou M, Grogan J, Mancewicz JA, Krausz KW, Gonzalez FJ, Gelboin HV, Korzekwa KR (1994) Activation of CYP3A4: evidence for the simultaneous binding of two substrates in a cytochrome P450 active site. *Biochemistry* 33:6450–6455
- Siller M, Goyal S, Yoshimoto FK, Xiao Y, Wei S, Guengerich FP (2014) Oxidation of endogenous N-arachidonoylserotonin by human cytochrome P450 2U1. *J Biol Chem* 289: 10476–10487
- Sipes RS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB (2013) Profiling 976 toxcast chemicals across 331 enzymatic and receptor signaling assays. *Chem Res Toxicol* 26:878–895
- Sladek NE, Mannering GJ (1969) Induction of drug metabolism. II. Qualitative differences in the microsomal N-demethylating systems stimulated by polycyclic hydrocarbons and by phenobarbital. *Mol Pharmacol* 5:186–199
- Sligar SG, Denisov IG (2007) Understanding cooperativity in human P450 mediated drug–drug interactions. *Drug Metab Rev* 39:567–579
- Sun P, Antoun J, Lin DH, Yue P, Gotlinger KH, Capdevila J, Wang WH (2012) Cyp2c44 epoxigenase is essential for preventing the renal sodium absorption during increasing dietary potassium intake. *Hypertension* 59:339–347
- Swan GE, Benowitz NL, Lessov CN, Jacob P 3rd, Tyndale RF, Wilhelmsen K (2005) Nicotine metabolism: the impact of CYP2A6 on estimates of additive genetic influence. *Pharmacogenet Genomics* 15:115–125
- Tamaki Y, Arai T, Sugimura H, Sasaki T, Honda M, Muroi Y, Matsubara Y, Kanno S, Ishikawa M, Hirasawa N, Hiratsuka M (2011) Association between cancer risk and drug-metabolizing enzyme gene (CYP2A6, CYP2A13, CYP4B1, SULT1A1, GSTM1, AND GSTT1) polymorphisms in cases of lung cancer in Japan. *Drug Metab Pharmacokinet* 26:516–522
- Thompson D, Oster G (1996) Use of terfenadine and contraindicated drugs. *JAMA* 275:1339–1341
- Tian Z, Cheng Q, Yoshimoto FK, Lei L, Lamb DC, Guengerich FP (2013) Cytochrome P450 107U1 is required for sporulation and antibiotic production in *Streptomyces coelicolor*. *Arch Biochem Biophys* 530:101–107

- Toide K, Yamazaki H, Nagashima R, Itoh K, Iwano S, Takahashi Y, Watanabe S, Kamataki T (2003) Aryl hydrocarbon hydroxylase represents *CYP1B1*, and not *CYP1A1*, in human freshly isolated white cells: trimodal distribution of Japanese population according to induction of *CYP1B1* mRNA by environmental dioxins. *Cancer Epidemiol Biomarkers Prev* 12:219–222
- Tyson CA, Lipscomb JD, Gunsalus IC (1972) The roles of putidaredoxin and P450<sub>cam</sub> in methylene hydroxylation. *J Biol Chem* 247:5777–5784
- Vaz ADN, Pernecky SJ, Raner GM, Coon MJ (1996) Peroxo-iron and oxenoid-iron species as alternative oxygenating agents in cytochrome P450-catalyzed reactions: switching by threonine-302 to alanine mutagenesis of cytochrome P450 2B4. *Proc Natl Acad Sci USA* 93:4644–4648
- Wang K, Guengerich FP (2012) Oxidation of fluorinated 2-aryl-benzothiazole antitumor molecules by human cytochromes P450 1A1 and 2W1. Deactivation by cytochrome P450 2S1. *Chem Res Toxicol* 25:1740–1751
- Waxman DJ, Holloway MG (2009) Sex differences in the expression of hepatic drug metabolizing enzymes. *Mol Pharmacol* 76:215–228
- Wedell A (2011) Molecular genetics of 21-hydroxylase deficiency. *Endocr Dev* 20:80–87
- West SB, Levin W, Ryan D, Vore M, Lu AYH (1974) Liver microsomal electron transport systems. II. The involvement of cytochrome *b<sub>5</sub>* in the NADH-dependent hydroxylation of 3,4-benzpyrene by a reconstituted cytochrome P-448-containing system. *Biochem Biophys Res Commun* 58:516–522
- White PC, New MI, Dupont B (1984) HLA-linked congenital adrenal hyperplasia results from a defective gene encoding a cytochrome P-450 specific for steroid 21-hydroxylation. *Proc Natl Acad Sci USA* 81:7505–7509
- Wienkers LC, Heath TG (2005) Predicting *in vivo* drug interactions from *in vitro* drug discovery data. *Nat Rev Drug Discov* 4:825–833
- Williams SN, Dunham E, Bradfield CA (2005) Induction of cytochrome P450 enzymes. In: Ortiz de Montellano PR (ed) *Cytochrome P450: structure, mechanism, and biochemistry*, 3rd edn. Kluwer Academic/Plenum, New York, pp 323–346
- Williams JA, Hyland R, Jones BC, Smith DA, Hurst S, Goosen TC, Peterkin V, Koup JR, Ball SE (2004) Drug–drug interactions for UDP-glucuronosyltransferase substrates: a pharmacokinetic explanation for typically observed low exposure (AUC<sub>i</sub>/AUC) ratios. *Drug Metab Dispos* 32:1201–1208
- Wu Z-L, Sohl CD, Shimada T, Guengerich FP (2006) Recombinant enzymes over-expressed in bacteria show broad catalytic specificity of human cytochrome P450 2W1 and limited activity of human cytochrome P450 2S1. *Mol Pharmacol* 69:2007–2014
- Xiao Y, Guengerich FP (2012) Metabolomic analysis and identification of a role for the orphan human cytochrome P450 2W1 in selective oxidation of lysophospholipids. *J Lipid Res* 53:1610–1617
- Xiao Y, Shinkyo R, Guengerich FP (2011) Cytochrome P450 2S1 is reduced by NADPH-cytochrome P450 reductase. *Drug Metab Dispos* 39:944–946
- Yamazaki H, Johnson WW, Ueng Y-F, Shimada T, Guengerich FP (1996) Lack of electron transfer from cytochrome *b<sub>5</sub>* in stimulation of catalytic activities of cytochrome P450 3A4: characterization of a reconstituted cytochrome P450 3A4/NADPH-cytochrome P450 reductase system and studies with apo-cytochrome *b<sub>5</sub>*. *J Biol Chem* 271:27438–27444
- Yamazaki H, Shimada T, Martin MV, Guengerich FP (2001) Stimulation of cytochrome P450 reactions by apo-cytochrome *b<sub>5</sub>*. Evidence against transfer of heme from cytochrome P450 3A4 to apo-cytochrome *b<sub>5</sub>* or heme oxygenase. *J Biol Chem* 276:30885–30891
- Yamazaki H, Komatsu T, Ohyama K, Nakamura M, Asahi S, Shimada N, Guengerich FP, Nakajima A, Yokoi T (2002) Roles of NADPH-P450 reductase and apo- and holo-cytochrome *b<sub>5</sub>* on xenobiotic oxidations catalyzed by 12 recombinant human cytochrome P450s expressed in membranes of *Escherichia coli*. *Protein Express Purif* 24:329–337
- Yang B, Graham L, Dikalov S, Mason RP, Falck JR, Liao JK, Zeldin DC (2001) Overexpression of cytochrome P450 CYP2J2 protects against hypoxia-reoxygenation injury in cultured bovine aortic endothelial cells. *Mol Pharmacol* 60:310–320



- Yang Q, Nagano T, Shah Y, Cheung C, Ito S, Gonzalez FJ (2008) The PPAR $\alpha$ -humanized mouse: a model to investigate species differences in liver toxicity mediated by PPAR $\alpha$ . *Toxicol Sci* 101:132–139
- Yang X, Zhang B, Molony C, Chudin E, Hao K, Zhu J, Gaedigk A, Suver C, Zhong H, Leeder JS, Guengerich FP, Strom SC, Schuetz E, Rushmore TH, Ulrich RG, Slatter JG, Schadt EE, Kasarskis A, Lum PY (2010) Genetic and genomic analysis of cytochrome P450 enzyme activities in human liver. *Genome Res* 20:1020–1036
- Yun C-H, Okerholm RA, Guengerich FP (1993) Oxidation of the antihistaminic drug terfenadine in human liver microsomes: role of cytochrome P450 3A4 in N-dealkylation and C-hydroxylation. *Drug Metab Dispos* 21:403–409
- Yun C-H, Kim K-H, Calcutt MW, Guengerich FP (2005) Kinetic analysis of oxidation of coumarins by human cytochrome P450 2A6. *J Biol Chem* 280:12279–12291
- Zhang H, Im S-C, Waskell L (2007) Cytochrome *b*<sub>5</sub> increases the rate of product formation by cytochrome P450 2B4 and competes with cytochrome P450 reductase for a binding site on cytochrome P450 2B4. *J Biol Chem* 282:29766–29776
- Zhao B, Guengerich FP, Bellamine A, Lamb DC, Izumikawa M, Funa N, Lei L, Podust LM, Sundaramoorthy M, Reddy LM, Kelly SL, Stec D, Voehler M, Falck JR, Moore BS, Shimada T, Waterman MR (2005) Binding of two flavin substrate molecules, oxidative coupling, and crystal structure of *Streptomyces coelicolor* A3(2) cytochrome P450 158A2. *J Biol Chem* 280:11599–11607
- Zhao B, Kagawa N, Sundaramoorthy M, Banerjee S, Nagy LD, Guengerich FP, Waterman MR (2012) A three-dimensional structure of steroid 21-hydroxylase (cytochrome P450 21A2) with binary substrate occupancy reveals locations of disease-associated variants. *J Biol Chem* 287:10613–10622

<http://www.springer.com/978-4-431-54991-8>

Fifty Years of Cytochrome P450 Research

Yamazaki, H. (Ed.)

2014, IX, 409 p. 101 illus., 51 illus. in color., Hardcover

ISBN: 978-4-431-54991-8